PhytoKeys 53: 1–25 (2015)
doi: 10.3897/phytokeys.53.4782
http://phytokeys.pensoft.net

RESEARCH ARTICLE

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Typification and taxonomic status re-evaluation of 15 taxon names within the species complex *Cymbella affinis/tumidula/turgidula* (Cymbellaceae, Bacillariophyta)

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Academic editor: *K. Manoylov* | Received 24 February 2015 | Accepted 2 July 2015 | Published 21 July 2015

Citation: da Silva WJ, Jahn R, Ludwig TAV, Hinz F, Menezes M (2015) Typification and taxonomic status re-evaluation of 15 taxon names within the species complex *Cymbella affinis/tumidula/turgidula* (Cymbellaceae, Bacillariophyta). PhytoKeys 53: 1–25. doi: 10.3897/phytokeys.53.4782

Abstract

Specimens belonging to the *C. affinis* / *C. tumidula* / *C. turgidula* species complex have many taxonomic problems, due to their high morphological variability and lack of type designations. Fifteen taxon names of this complex, distributed in five species, were re-evaluated concerning their taxonomic status, and lectotypified based on original material. In addition to light microscopy, some material was analyzed by electron microscopy. Four new combinations are proposed in order to reposition infraspecific taxa.

Keywords

Diatoms, typification, Cymbellales, *Cymbella tropica*, *Cymbella subturgidula*, *Cymbella rheophyla*, *Cymbella uenoi*

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Introduction

The history of the genus *Cymbella* C.Agardh is replete with taxonomic complexities. Within these complexes many species are similar in valve morphology. Much of the confusion in these complexes was caused by poor species descriptions including specimen images and the lack of designation of types, which has been required by the International Code of Nomenclature for algae, fungi, and plants (ICN) only since 1958 (McNeill et al. 2012).

In the most recent revision of the genus *Cymbella*, Krammer (2002) characterized and emended species descriptions within the complex *C. affinis* Kütz. / *C. tumidula* Grunow / *C. turgidula* Grunow. This has generated confusion in the taxonomy of the group, specifically concerning the typification of *C. affinis*. The concept of *C. affinis* as proposed by Krammer (2002) involved the synonymization of *C. affinis* and *C. tumidula* Grunow, and the restoration of *C. excisa* Kütz. *Cymbella excisa* has previously been treated by some authors as a synonym of *C. affinis*, at the same or at an infraspecific rank (e.g. Cleve 1894, Grunow 1882, Patrick and Reimer 1975). According to Krammer (2002), the specimens treated as *C. affinis* have cymbelloid outlines, with the axial area straight to slightly curved and a prominent central area. In these specimens the valvar ends are more protracted and more densely striated and areolated than in *C. excisa*, which has the axial area curved and an indistinct central area. Moreover, *C. excisa* commonly has an excision in the ventral middle part of the valve.

Krammer in Krammer and Lange-Bertalot (1986) also designated a neotype for *C. turgidula*, a species with an outline very similar to *C. affinis* but with wider, fewer and uniformly distributed striae, and less-dense punctae in the striae. In the same contribution, Krammer also described the new species *C. subturgidula* Krammer, which he distinguished from *C. turgidula* by its narrower breadth, higher length/breadth ratio, and the size and shape of the central area.

Tuji (2007) designated a lectotype for *C. affinis* from an original illustration provided by Kützing (1844, Pl. 6, Fig. 15). Moreover, he assigned an epitype to a single specimen in preparation BM 18530 (Tuji 2007, Fig. 9) which was made from the original sample, which is Kützing's packet 333.

Tuji (2007) observed that the designation of a neotype for *C. turgidula* by Krammer in Krammer and Lange-Bertalot (1986) was inappropriate. This designation was made because Krammer was unable to locate the original samples or preparations (Krammer and Lange-Bertalot 1986). Tuji (2007) found the original slide (syntype) used by Grunow in Schmidt (1875) to describe *C. turgidula*, and designated an original illustration as a lectotype and one specimen on Grunow's slide as an epitype.

The material of *C. uenoi* Skvortsov was also revisited by Tuji (2007), which according to him would make *C. subturgidula* a synonym. He also proposed the new combination *C. uenoi* f. *nipponica* (Skvortsov) Tuji for *C. turgidula* var. *nipponica* Skvortsov [≡ *Cymbella rheophila* Ohtsuka].

Despite the changes, the revision of Tuji (2007) did not resolve the status of taxa such as *C. tumidula* or *C. excisa*. Moreover, he overlooked some requirements by the ICN with respect to the validity of the name *C. uenoi*.

The aim of the present study was to elucidate the current taxonomic status of *C. tumidula*, *C. excisa* and *C. subturgidula*, as well as of *C. uenoi* and *C. uenoi* f. *nipponica* [≡ *C. turgidula* var. *nipponica*; ≡ *Cymbella rheophila*]; to revisit the infraspecific taxa encompassed in this complex of species; and to lectotypify the ambiguous taxa.

Materials and methods

We analyzed the protologues and morphological features from materials of *C. affinis* var. *affinis*, *C. affinis* var. *procera*, *C. excisa* var. *excisa*, *C. excisa* var. *procera*, *C. excisa* var. *angusta*, *C. excisa* var. *subcapitata*, *C. tumidula* var. *tumidula*, *C. salinarum*, *C. turgida*, *C. tropica*, *C. subturgidula*, *C. turgidula* var. *nipponica*, *C. reophila*, *C. uenoi* f. *uenoi*, and *C. uenoi* f. *nipponica* (Table 1). The characterization of *C. salinarum* and *C. uenoi* f. *uenoi* was based on the illustration of the holotype provided by Krammer (2002, Fig. 25: 13) and of the epitypes illustrated by Tuji (2007, Figs 14–19).

Materials of *C. affinis* (BM18530), *C. excisa* (BM 18543), *C. tumidula* (BM 18543), *C. turgidula* var. *nipponica* (R 214.928, R 214.929, R 214.930, R 214.931 and R 214.932) were analyzed in the Laboratório de Ficologia, Museu Nacional, Rio de Janeiro, Brazil, using an Olympus BX 51 microscope (Olympus, Tokyo, Japan) fitted with an Olympus Q-Color digital camera. Images were processed with Q capture Pro QImaging© software.

Materials of *C. affinis* sensu Krammer (1198F IOK BRM) and *C. affinis* var. *procera* (714 IOK BRM) were examined in the laboratory of the Botanischer Garten und Botanisches Museum (BGBM), Berlin, Germany, using a Zeiss Axio Imager 4.2 microscope (Carl Zeiss MicroImaging GmbH, Berlin), and the images were captured through an MRc/MRm system (Carl Zeiss MicroImaging) and the software AxioVision Rel. 4.8 (Carl Zeiss MicroImaging).

We also analyzed materials *C. excisa* sensu Krammer (1131G IOK BRM), *C. excisa* var. *procera* (212A IOK BRM), *C. excisa* var. *angusta* (752 IOK BRM), *C. excisa* var. *subcapitata* (717A IOK BRM), *C. tropica* (1015D IOK BRM) and *C. subturgidula* (1046E IOK BRM) with a Zeiss Axioplan microscope (Carl Zeiss, Jena, Göttingen, Germany) with an Olympus XC50 capture system (Olympus, Tokyo, Japan) and the software analySIS Image Processing (Soft Imaging System, Münster, Germany), at the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany.

Scanning Electron Microscopy (SEM) analyses were carried out only for *C. turgidula* var. *nipponica*. Samples were deposited on cover slips and attached to aluminum stubs using LeitSilver® (Sigma-Aldrich, Berlin, Germany). The material was coated with 150–200 Å of gold in an Emitech K 550 sputter coater (Quorum Technologies Ltd., Kent, UK). The material was analyzed in a Jeol JSM-6390 scanning electron microscope (Jeol, USA), operated at 6–8 kV, spot size 10–30, in the electron microscopy laboratory in the Museu Nacional, Rio de Janeiro, Brazil.

The term "degree of dorsiventrality" is used here to define how dissimilar the sides of the valvae are on the apical axis. The symbols "≡", "=" and "–" preceding

Table 1. Metric features of the material of the complex *C. affinis*/*tumidula*/*turgidula* analyzed in this study (*n*= 29–34 individuals).

Taxon (published name)	Current name	Material	Length (µm)	Breadth (µm)	L/B ratio	Striae in middle part (in 10 µm)	Striae close to the ends (in 10 µm)	Puncta (in 10 µm)	Stigmata
C. affinis Kütz. sensu Krammer (2002)	*C. tumidula* Grunow var. *tumidula*	Preparation 1198F IOK in BRM, from France, Normandy, Falaise, coll. Brébisson, in BRM (Figs 50–56)	25.4–29.8	7.8–8.7	3.4–3.9	10–15	12–19	26–33	2–3
C. affinis Kützing (1844)	*C. affinis* Kützing var. *affinis*	Lectotype designated by Tuji (2007), preparation BM 18530, from France, Falaise, coll. Brébisson, in BM (Figs 1–7)	22.5–26.7	7.0–8.5	2.9–3.4	9–12	11–18	19–27	1
C. affinis var. *procera* Krammer (2002)	*C. tumidula* var. *procera* (Krammer) W.Silva, comb. nov.	Holotype, preparation 714 IOK, from Serbia, Rogatica, 1976, in BRM (Figs 57–63)	27.0–40.3	9.0–10.2	2.9–4.2	9–13	13–18	25–31	2–5
C. excisa var. *procera* Krammer (2002)	*C. affinis* var. *neoprocera* W.Silva, comb. nov. et nom. nov.	Holotype, preparation 212A IOK, from West Germany, Eifel, Totenmaar, 22.2.1974, in BRM (Figs 22–28)	21.5–41.0	8.0–11.0	2.7–4.0	9–13	11–14	22–27	1
C. excisa Kützing (1844)	*C. affinis* var. *excisa* (Kütz.) Grunow	Isolectotype, preparation 1131G IOK, Hauck No. 72 from Italy, 26.3.1837, in BRM (Figs 15–21)	23.1–28.5	7.0–9.5	2.7–3.9	9–12	11–17	24–26	1
C. excisa var. *angusta* Krammer (2002)	*C. affinis* var. *angusta* (Krammer) W.Silva, comb. nov.	Holotype, preparation 752 IOK, from Croatia, Lake Gavanovac, 18.5.1976, in BRM (Figs 29–35)	20.5–35.0	6.0–8.5	3.2–4.6	9–15	11–18	19–29	1
C. excisa Kützing (1844)	*C. affinis* var. *excisa* (Kütz.) Grunow	Lectotype (designated here), preparation BM 18543, from Hauk No. 72 from Italy, 26.3.1837, in BM (Figs 8–14)	21.5–26.5	7.0–8.7	2.6–3.5	9–13	11–16	21–25	1
C. excisa var. *subcapitata* Krammer (2002)	*C. affinis* var. *subcapitata* (Krammer) W.Silva, comb. nov.	Holotype, preparation 717A IOK, from Hungary, Balaton, 6.77, in BRM (Figs 36–42)	21.0–31.7	7.4–9.0	2.7–4.0	8–12	9–15	26–31	1

Taxon (published name)	Current name	Material	Length (µm)	Breadth (µm)	L/B ratio	Striae in middle part (in 10 µm)	Striae close to the ends (in 10 µm)	Puncta (in 10 µm)	Stigmata
C. subturgidula Krammer (2002)	*C. subturgidula* Krammer	Lectotype, preparation 1046E IOK, from Korea, Ulchin County, Kyungsang Pukdo, Kwangchun River, in BRM (Figs 71–77)	30.3–37.4	9.0–12.8	2.6–3.5	9–11	12–15	21–24	2
C. tropica Krammer (2002)	*C. tropica* Krammer	Holotype, preparation 1015D IOK, from Venezuela, Rio Manizanes, coll. Rumrich 4.4.1990, in BRM (Figs 64–70)	34.5–42.7	10.0–12.0	3.1–4.0	9–12	11–12	21–24	1
C. tumidula Grunow in Schmidt (1875)	*C. tumidula* Grunow var. *tumidula*	Lectotype (designated here); Epitype (designated here), preparation BM 18543, from Hauck No. 72, from Italy, 26.3.1837, in BM (Figs 43–49)	26.8–34.7	7.8–8.7	3.4–4.0	10–15	12–19	26–33	2–4
C. salinarum Grunow in Schmidt (1875)	*C. tumidula* var. *salinarum* (Grunow) Cleve	Holotype illustration provided Krammer (2002, Fig. 25: 13)	34.0	10.7	3.2	14	15	–	1
C. turgidula Grunow in Schmidt (1875)	*C. turgidula* Grunow	Epitype designated by Tuji (2007), preparation 1504, from Puerto Rico, in the Grunow Collection, in W	36.5–45.0	10.8–13.3	3.2–3.4	9–12	11–17	21–24	1–3
C. turgidula var. *nipponica* Skvortsov (1936) [≡ *C. rheophila* Ohtsuka in Ohtsuka and Tuji (2002); ≡ *C. uenoi* f. *nipponica* (Skvortsov) Tuji (2007)]	*C. subturgidula* Krammer	Isolectotype, preparation R 214.928, from sample 0983 from Japan, Lake Biwa, coll. Tamiji Kawamura, 03.11.1915, in R (Figs 78–80)	28.1–39.0	10.0–13.2	2.8–3.0	9–12	12–15	21–26	1–2
		Preparations R 214.929 and R 214.930, from sample 1062, from Japan, Yamanoshita Bay in Lake Biwa, Otsu City, Shiga Prefecture, coll. Yasuko Iwao in 23.01.1993, in R (Fig. 81)	27.7–39.2	10.3–13.1	2.5–3.6	9–12	12–15	22–26	1–2

Taxon (published name)	Current name	Material	Length (µm)	Breadth (µm)	L/B ratio	Striae in middle part (in 10 µm)	Striae close to the ends (in 10 µm)	Puncta (in 10 µm)	Stigmata
		Preparations R 214.931 and R214.932, from from sample 1093, from Japan, cobble in Lake Biwa, at Uchidehama, Otsu City, Shiga Prefecture, coll.Yasuko Iwao, 03.03.1993, in R (Figs 82–84)	26.5–40.1	11.1–13.5	2.3–3.4	9–13	12–14	21–24	2–3
C. uenoi Skvortsov *ex*Tuji (2007)		Epitype and isotype illustrations provided Tuji (2007, Figs 21–24, 26)	26.3–41.0	9.0–12.7	2.9–3.2	9–11	12-15	21–24	2

specific and infraspecific names are used to represent homotypic or nomenclatural, heterotypic or taxonomic, and concept synonyms, respectively, as used in the ICN (McNeill et al. 2012).

Results

Cymbella affinis Kütz

Figs 1–7

Cymbella affinis Kütz., Bacill., 80, Pl. 6 Fig. 15, 1844.

Lectotype. 3rd figure from the left in Kützing (1844, pl. 6, Fig. 15) designated by Tuji (2007).

Type locality. "Falaise (France): De Brébisson! Herb. Binder., Schleswig: Herb. Binder.!"

Epitype. An individual on preparation BM18530, from Kützing packet 333, in the Natural History Museum (BM), designated by Tuji (2007, Fig. 9).

Epitype locality. Falaise, France, coll. De Brébisson.

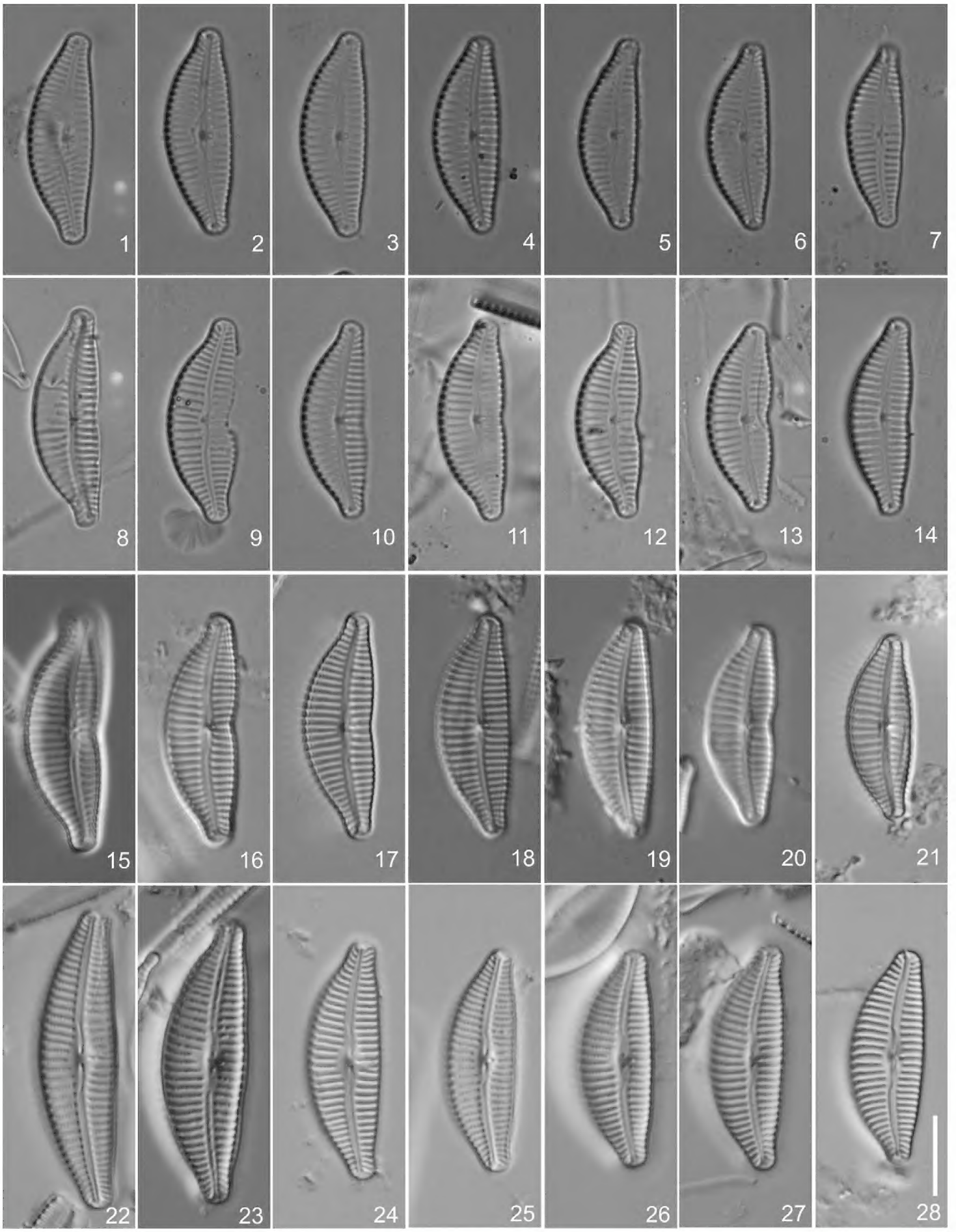
Valvae lanceolate, dorsiventral, dorsal and ventral margins convex; ends barely protracted, rounded, to slightly subrostrate or subcapitate; length 22.5–26.5 μm, breadth 7.0–8.5 μm, L/B ratio 2.9–3.4; axial area narrow, linear-arched, indistinct central area; striae 9–12 in 10 μm, becoming 11–18 towards ends, one isolated pore at end of central striae on ventral side; 19–27 punctae in 10 μm.

Remarks. Although Krammer (2002, p. 41) used material sampled by the same collector and from the same locality and related his new preparation to the type locality, his effort did not constitute a typification of *C. affinis* as ruled by the ICN (Art. 7.10, McNeill et al. 2012). On the other hand, Tuji (2007) was the first to consider the original material of this taxon and performed a lectotypification and epitypification, and as such, must be followed according to Articles 9.19 and 9.20 (McNeill et al. 2012).

The lectotype valve of *C. affinis* is similar to the lectotype valve of *C. excisa*, except for the excision in the middle part of the valve present in the majority of specimens (Figs 8–21). Populations of this complex from different parts of the world may or may not have excisions, but this character is present in the majority of specimens from the populations examined (Krammer 2002). Therefore, we consider *C. excisa* and *C. affinis* as belonging to the same species with differences at the varietal rank based on phenotypic expression and ecological modifications. Genetic studies are still to be completed.

The two taxa were both proposed by Kützing (1844, p. 80) and therefore have the same priority. In similar cases, Article 11.5 of the ICN rules that "the first such choice to be effectively published establishes the priority of the chosen name".

Grunow (1882) proposed the new combination and the new status of *C. excisa* for *C. affinis* var. *excisa* (Kütz.) Grunow. This was the first publication that defined



Figures 1–28. *Cymbella affinis* species complex **1–7** *C. affinis* Kütz. var. *affinis* **8–14** *C. affinis* var. *excisa* (Kütz.) Grunow, specimens from Trieste in preparation BM 18543 **11** Lectotype, designated here **15–21** *C. affinis* var. *excisa* [– *C. excisa* sensu Krammer], specimens from Trieste in preparation 1131 IOK **16** Isolectotype, designated here **22–28** *C. affinis* var. *neoprocera* W.Silva, specimens from Germany in preparation 212A IOK, holotype **22** Lectotype, designated here. Scale bar: 10 μm.

the priority of the epithet *affinis* over *excisa* at the specific level. Therefore, the name *C. affinis* must be considered to be the name of the species when *C. excisa* and *C. affinis* are considered to be the same species, in conformity with Article 11.5 of the ICN (McNeill et al. 2012).

Cymbella affinis valves sensu Patrick and Reimer (1975, p. 57) have similar outlines as *C. affinis* in the type population, but higher range of length and breadth values (length: 20–50 vs. 22.5–26.5 µm; breadth: 7–12 vs. 7.0–8.3 µm, respectively). However, these authors (Patrick and Reimer 1975), included representatives of different localities that could encompass different varieties subscribed to this taxon. The density of striae in the material from USA was similar in the middle part of the valve compared to the type material (9–11 vs. 9–12 striae in 10 µm, respectively) and lower when comparing them close to the ends of the valvae (12–14 vs. 11–18 striae in 10 µm, respectively) (Patrick and Reimer 1975).

C. excisa var. *procera* Krammer (Figs 22–28), *C. excisa* var. *angusta* Krammer (Figs 39–35) and *C. excisa* var. *subcapitata* Krammer (Figs 36–42) also appear to be conspecific with *C. affinis*, but the types show slight differences in their outlines and metric characteristics (Table 1). Thus, all these taxa are here transferred to *C. affinis* using their respective infraspecific epithet.

***Cymbella affinis* var. *excisa* (Kütz.) Grunow**

Figs 8–21

Cymbella affinis var. *excisa* (Kütz.) Grunow, Beitr. Paläont. Österreich.-Ungarns Orients, 2: 142, Pl. 19(1), Fig. 26, 1882.

Basionym. *Cymbella excisa* Kütz., Bacill., 80, Pl. 6, Fig. 17, 1844.

Lectotype (designated here). An individual marked with blue ring on preparation BM 18543, from Hauck No. 72, 26.4.1837, in the Natural History Museum (BM), London, United Kingdom, represented by Fig. 11.

Isolectotype (designated here). An individual on preparation 1131G IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany, represented by Fig. 16.

Type locality. "Unter Oscillatorien in Bächen bei Triest", Italy, 26.4.1837.

Valvae dorsiventral, dorsal margin broadly convex, ventral margin straight, usually with an excision in middle portion; ends subrostrate to rostrate; length 21.5–28.5 µm, breadth 7.0–9.5 µm, L/B ratio 2.6–3.9; axial area narrow, linear-arched, central area indistinct; striae 9–13 in 10 µm, becoming 11–17 toward ends, one isolated pore at end of central striae on ventral side; 21–26 punctae in 10 µm.

Remarks. Similarly to *C. affinis*, the lectotypification of *Cymbella excisa* designated by Krammer (2002, p. 26) cannot be considered according to Article 7.10 of the ICN

(McNeill et al. 2012), because the phrase "designated here" or equivalent is required from 1 January 2001 and it was not included by Krammer (2002). Therefore, the lectotype designated by us cannot be considered a replacement of Krammer's (2002) "lectotype"; rather, it is the first lectotypification of this taxon.

The main difference between *C. affinis* var. *excisa* and the nominate variety is the presence of an excision in the middle portion of the ventral side of the valve, a characteristic common to populations of this taxon around the world (Krammer 2002). Therefore, we consider that the presence of excisions in all populations, not present in the type material of *C. affinis*, constitutes sufficient grounds to consider *C. affinis* var. *excisa* different from the nominate variety, which conforms to the statement by Grunow (Schmidt 1875); and not a different species.

Krammer (2002) recorded populations in the isotype material of *C. affinis* var. *excisa* with length 17–41 µm, and breadth 6.0–10.7 µm, which were higher than populations in the lectotype material. Krammer (2002) was able to observe initial and post initial cells which were similar to the minimum and maximum length and breadth of this taxon. However, Krammer (2002) included specimens of the variety *excisa* and the nominate variety in his description.

***Cymbella affinis* var. *neoprocera* W.Silva, comb. nov. et nom. nov.**
Figs 22–28

Basionym. *Cymbella excisa* var. *procera* Krammer, Diatoms Europe 3: 159, Figs 9:1–7, 2002 (Figs 22–28).

Holotype. Preparation 212A IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany.

Lectotype (designated here). An individual on preparation 212A IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany; represented by Fig. 22.

Type locality. Germany, Eifel, Totenmaar, 22.2.1974.

Valvae dorsiventral, dorsal margin broadly convex, ventral margin straight; ends not protracted, rounded, or subrostrate to rostrate; length 21.5–41.0 µm, breadth 8.0–11.0 µm, L/B ratio 2.7–4.0; axial area narrow, linear-arched, indistinct central area; striae 9–13 in 10 µm, becoming 11–14 toward ends, one isolated pore at end of central striae on ventral side; 22–27 punctae in 10 µm.

Remarks. The combination of *C. excisa* var. *procera* with *Cymbella affinis* would be illegitimate unless it was given a new name, because it would be a later homonym of *C. affinis* var. *procera* Krammer. Specimens designated by Krammer (2002), i.e., the preparation 212A IOK (BRM), were found to belong to more than one taxon therefore to clarify the taxonomy we designated a lectotype as established in Art. 9.11 of the ICN (McNeill et al. 2012).

A broader range of metric characteristics were highlighted in this study compared to the characterization of the type population (Krammer 2002, p. 28). We find smaller

(21.5–41.0 vs. 24–41 μm) and narrower (8.0–11.0 vs. 8.4–11.0 μm) representatives of this taxon compared to the Krammer's (2002) records. Still considering Krammer's (2002) findings, we observe more densely striated (9–13 vs. 9–11 striae in 10 μm) and less punctate (22–27 vs. 24–27 punctae in 10 μm) valves.

***Cymbella affinis* var. *angusta* (Krammer) W.Silva, comb. nov.**
Figs 29–35

Basionym. *Cymbella excisa* var. *angusta* Krammer, Diatoms Europe 3: 159, Figs 9: 8–13, 2002.

Holotype. Preparation 752 IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany.

Lectotype (designated here). An individual on preparation 752 IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany, represented by Fig. 29.

Type locality. Croatia, watercourse near Lake Gavanovac, Plitvice.

Valves dorsiventral, dorsal margin broadly convex, ventral margin straight, usually with excision in middle portion; ends not protracted to slightly protracted, rounded, subrostrate or subcapitate; length 20.5–35.0 μm, breadth 6.0–8.5 μm, L/B ratio 3.2–4.6; axial area linear-arched, indistinct central area; striae 9–15 in 10 μm, becoming 11–18 towards ends, one isolated pore at end of central striae of ventral side; 19–29 punctae in 10 μm.

Remarks. The material recorded here presented slight differences in the valve metrics compared to Krammer's (2002, p. 28) characterization from the same preparation; differences included valve length (20.5–35.0 vs. 17.0–34.0 μm), breadth (6.0–8.4 vs. 6.7–8.2 μm), striae (9–15 vs. 11–14 in 10 μm) and number of punctae (19–29 vs. 25–28 in 10 μm).

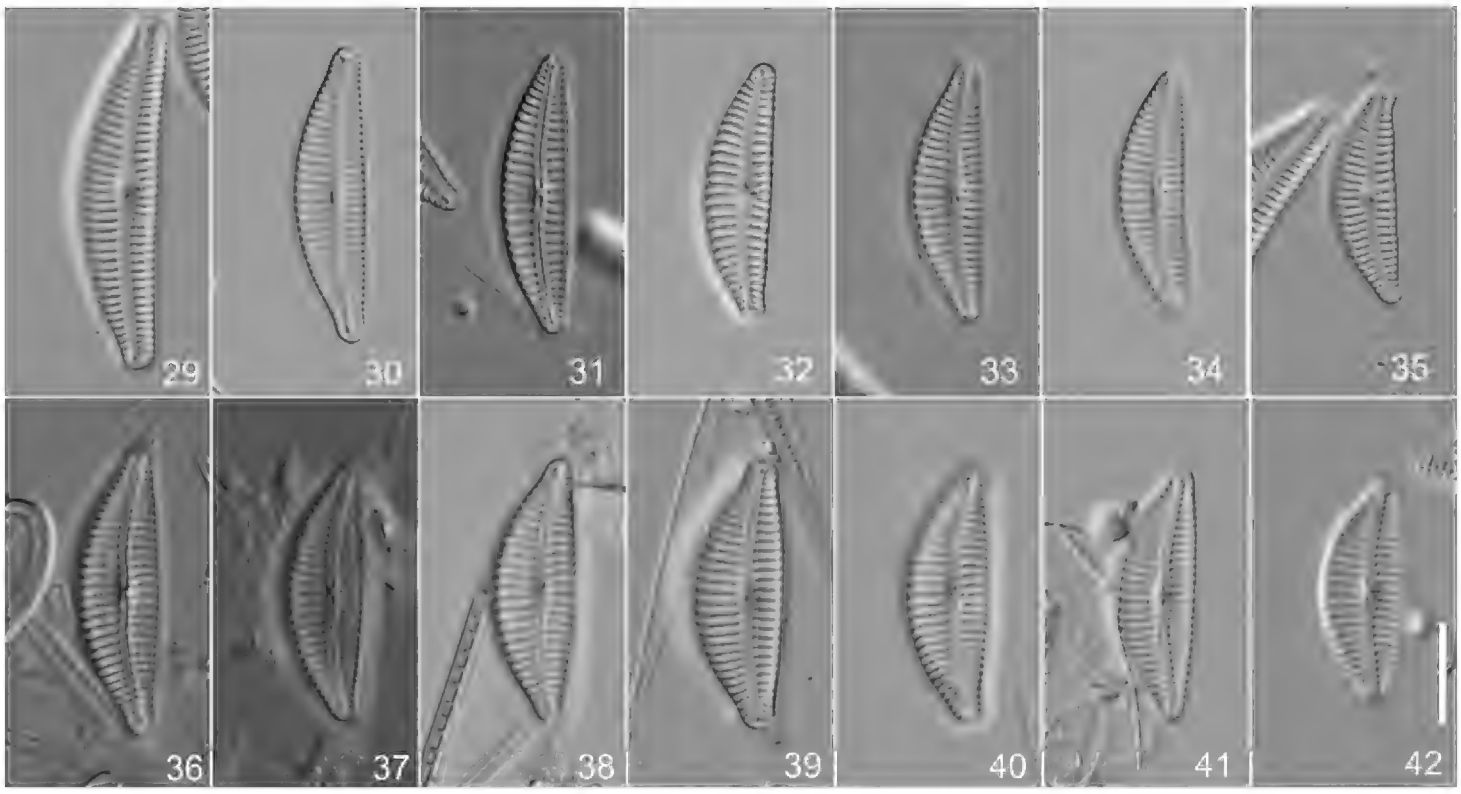
Similar to *C. affinis* var. *neoprocera*, specimens designated by Krammer (2002), i.e., the preparation 752 IOK (BRM), were found to belong to more than one taxon. Therefore we designated a lectotype for this taxon as established in Art. 9.11 of the ICN (McNeill et al. 2012).

***Cymbella affinis* var. *subcapitata* (Krammer) W.Silva, comb. nov.**
Figs 36–42

Basionym. *Cymbella excisa* var. *subcapitata* Krammer, 2002, Diatoms of Europe 3: 159, Figs 10: 14–18.

Holotype. Preparation 717A IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany.

Lectotype (designated here). An individual on preparation 717A IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany, represented by Fig. 39.



Figures 29–42. *Cymbella affinis* species complex **29–35** *C. affinis* var. *angusta* (Krammer) W.Silva, specimens from Croatia in preparation 752 IOK, holotype **29** Lectotype, designated here **36–42** *C. affinis* var. *subcapitata* (Krammer) W.Silva, specimens from Hungary in preparation 717 IOK, holotype **39** Lectotype, designated here. Scale bar: 10 µm.

Type locality. Hungary, Balaton.

Valves dorsiventral, dorsal margin broadly convex, ventral margin straight, usually with excision in middle portion; ends barely protracted, rounded, to broadly protracted, subcapitate; length 21.0–31.7 µm, breadth 7.4–9.0 µm, L/B ratio 2.7–4.0; axial area linear-arched, indistinct central area; striae 8–12 in 10 µm, becoming 9–15 towards ends, one isolated pore at end of central striae of ventral side; 26–31 punctae in 10 µm.

Remarks. Krammer (2002) distinguished this variety from the variety *excisa* based on the shape of the ends of the valvae. However, he (Krammer 2002, p. 28) did not provide metric characterizations of this the variety *subcapitata*, which we observed to agree with the characterizations of *C. excisa* sensu Krammer (2002), except for the density of striae that was slightly lower in the variety *subcapitata*.

Specimens designated by Krammer (2002), i.e., the preparation 717A IOK (BRM), were found to belong to more than one taxon. Therefore we designated a lectotype as established in Art. 9.11 of the ICN (McNeill et al. 2012).

Cymbella tumidula Grunow var. *tumidula*

Figs 43–56

Cymbella tumidula Grunow var. *tumidula*, in Schmidt, A. Schmidt's Atlas Diatom.-Kunde, Pl. 9, Fig. 33, 1875.

Synonym. *Cymbella affinis* Kütz. *sensu* Krammer, Diatoms Europe 3: 41, Figs 21:2–21, 22:1–20, 23:1–18, 2002. (Figs 50–56).

Lectotype (designated here). Plate 9, Fig. 33 from Schmidt (1875a) (Fig. 43).

Type locality. Italy, Trieste, 26.4.1837.

Epitype (designated here). An individual marked with a red ring on preparation BM 18543 from Hauck No. 72, 26.4.1837, in the Natural History Museum (BM), London, United Kingdom, represented by Fig. 49.

Isoepitype (designated here). An individual on preparation 1131G IOK, from Hauck No. 72, 26.4.1837, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany, represented by Fig. 54.

Epitype locality. Italy, Trieste, 26.4.1837.

Valves lanceolate, dorsiventral, dorsal and ventral margins convex; subcapitate ends; length 25.4–34.7 μm, breadth 7.8–8.7 μm, L/B ratio 3.4–4.0; axial area linear-lanceolate, straight to arched, central area irregular to rounded; striae 10–15 in 10 μm, becoming 12–19 toward ends, 2–4 isolated pores at end of central striae on ventral side; 26–33 punctae in 10 μm.

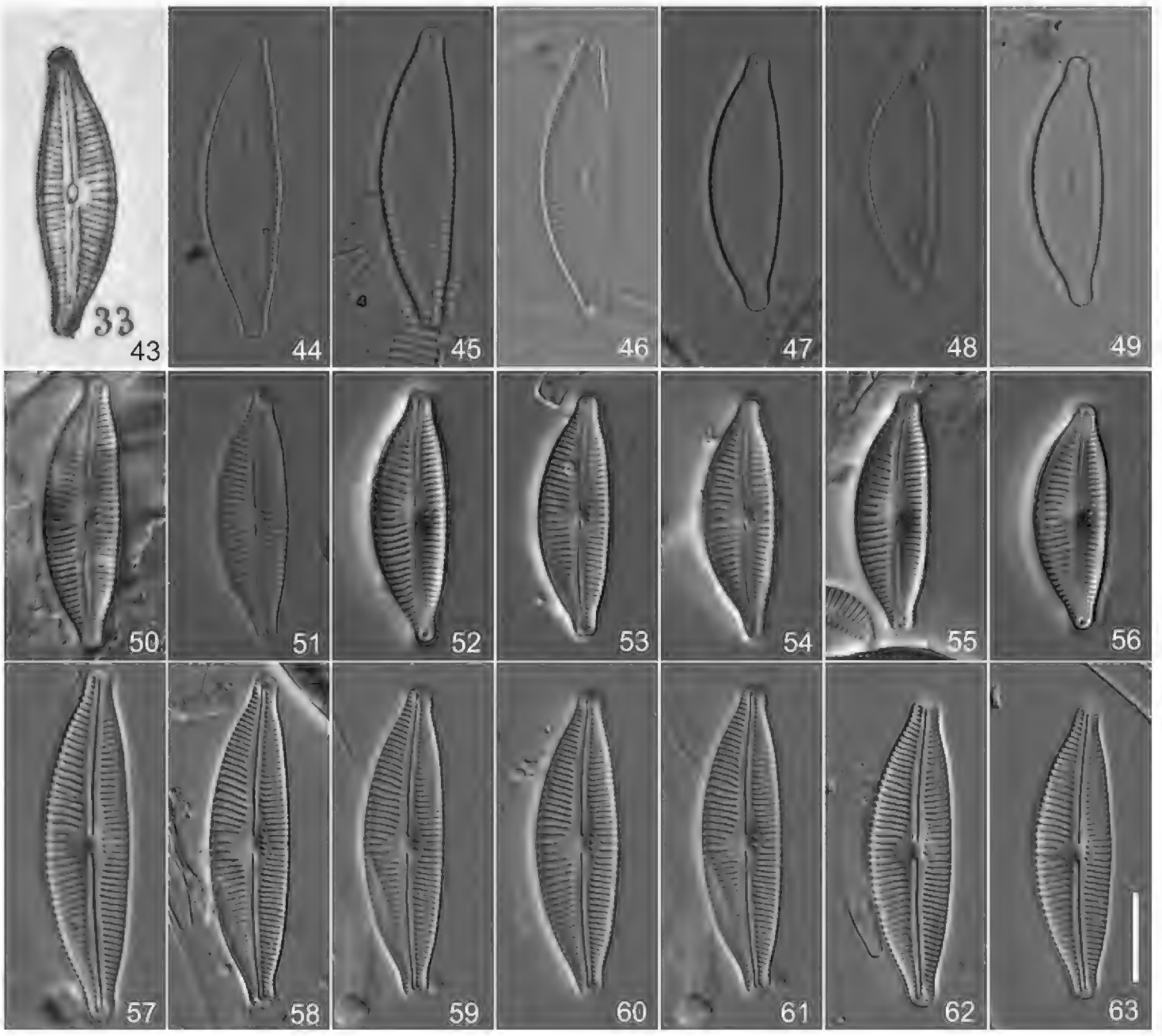
Remarks. The lectotypification of *C. affinis* allowed us to consider *C. affinis* and *C. tumidula* [– *C. affinis* sensu Krammer], lectotypified and epitypified here, as independent species. *C. tumidula* has a more lanceolate outline, subcapitate ends, and a lower degree of dorsiventrality than *C. affinis*. The striae in the middle part of the valve are shorter and unevenly distributed in *C. tumidula*, forming a distinct central area (Figs 43–63), in contrast to *C. affinis* where the central area is indistinct and the striae are uniformly distributed. In addition, all specimens of the type material of *C. affinis* have only one stigma, whereas in *C. tumidula* 1–5 stigmata can be observed.

Specimens from the population of the holotype material of *C. affinis* var. *procera* were very similar in outline but larger and wider than *C. tumidula* var. *tumidula*, resulting in higher maximum length/breadth ratios. However, all metric characteristics of *C. affinis* var. *procera* intergraded with *C. tumidula* var. *tumidula*, and therefore this taxon was transferred to *C. tumidula* var. *procera*.

The characteristics of *C. affinis* sensu Krammer (2002, p. 41) were similar to the type population of *C. tumidula*, but with a wider range of values. This includes length (17–34 vs. 25.4–34.7 μm, respectively) and breadth (7.5–9.5 vs. 7.8–8.7 μm). *C. affinis* sensu Krammer (2002, p. 41) also presented narrower range of values of striae in 10 μm (10–13, becoming 13–15 toward ends vs. 10–15 in 10 μm, becoming 12–19 toward ends, respectively) and density of punctae (27–32 vs. 26–33 punctae in 10 μm) than *C. tumidula*.

***Cymbella tumidula* var. *procera* (Krammer) W.Silva, comb. nov.**
Figs 57–63

Basionym. *Cymbella affinis* var. *procera* Krammer, Diatoms Europe 3: 161, Figs 22: 8–13, 2002.



Figures 43–63. *Cymbella tumidula* species complex **43** Original illustration provided by Grunow in Schmidt (1875, Pl. 9, Fig. 33), lectotype, here designated **44–49** *C. tumidula* var. *tumidula*, specimens from Trieste in preparation BM 18543 **49** Epitype, here designated **50–56** *C. tumidula* var. *tumidula* [*C. affinis* sensu Krammer (2002)], specimens from Trieste in preparation 1131G IOK **54** Isolectotype, designated here **57–63** *C. tumidula* var. *procera* (Krammer) W.Silva, specimens from Serbia in preparation 714 IOK, holotype **57** Lectotype, designated here. Scale bar: 10 μm.

Holotype. Preparation 714 IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany.

Lectotype (designated here). An individual on preparation 714 IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany, represented by Fig. 57.

Type locality. Serbia, Rogatica (abundant in chalk-rich spring), 1976.

Valves lanceolate, dorsiventral, dorsal and ventral margins convex; ends subrostrate or subcapitate; length 27.0–40.3 μm, breadth 9.0–10.2 μm, L/B ratio 2.9–4.2; axial area linear-lanceolate, slightly arched, central area rounded; striae 9–13 in 10 μm, becoming 13–18 toward ends, 2–5 isolated pores; 25–31 punctae in 10 μm.

Remarks. According to Krammer (2002), this variety differs concerning wider valves (higher than 9.5 μm) from the nominate variety of *C. affinis* sensu Krammer. We recorded specimens of *C. tumidula* var. *procera* with 9 μm breadth, which were higher than the nominate variety (9.0–10.2 vs. 7.8–8.7 μm).

Specimen designated by Krammer (2002), i.e., the preparation 714 IOK (BRM), were found to belong to more than one taxon. Therefore we designated a lectotype as established in Art. 9.11 of the ICN (McNeill et al. 2012).

Cymbella tumidula var. *salinarum* Grunow

Cymbella tumidula var. *salinarum* (Grunow) Cleve, Kongl. Svenska Vetensk.-Akad. Handl., ser. 4, 26(2): 171, 1894.

Basionym. *Cymbella salinarum* Grunow in Schmidt, A. Schmidt's Atlas Diatom.-Kunde, Pl. 9, fig. 28, 1875.

Holotype. Preparation 1603 in the Grunow Collection in the Naturhistorisches Museum Wien (W).

Lectotype (designated here). An individual on preparation 1603, in the Grunow Collection in the Naturhistorisches Museum Wien (W), represented by the illustration in Krammer (2002, Fig. 25: 13).

Type locality. Salinen von Zaule (Trieste, Italy).

Valves lanceolate, dorsiventral, dorsal and ventral margins convex; ends subcapitate; length 34.0 μm, breadth 10.7 μm, L/B ratio 3.2; axial area linear-lanceolate, slightly arched, central area rounded; striae 14 in 10 μm, becoming 15 toward ends, 1 isolated pore.

Remarks. This taxon presents morphometric characteristics similar to *C. tumidula* var. *tumidula*, except it has wider valves. Cleve (1894) recorded specimens of *C. tumidula* var. *salinarum* with 27–40 μm length, 8–10 μm breadth, and 11 or 12 striae in 10 μm, and considered that the only difference between this taxon and *C. tumidula* var. *tumidula* was the shape of the ends. Although in poor condition, in preparation 1603 we did not find differences between the shape of the valvar ends of the variety *salinarum* and the nominate variety. However, *C. tumidula* var. *salinarum* has higher breadth values compared to the type population of *C. tumidula* var. *tumidula*, even in populations of this taxon as recorded by Krammer (2002) from Falaise where initial and post initial cells were found. Moreover, the occurrence of *C. tumidula* var. *salinarum* has been restricted to brackish waters.

Krammer (2002, Fig. 25: 13) provided the illustration of an individual of the type of *C. salinarum*. The individual represented by him (Krammer 2000) was similar to *C. tumidula* var. *tumidula*. However, it was larger and had only one isolated pore, differing from *C. tumidula*, which has more than two isolated pores (Figs 50–56). Thus, in contrast to Krammer (2002), who treated *C. salinarum* at the specific level, we consider this taxon at the infraspecific rank as did Cleve (1894).

Cymbella turgidula Grunow

Cymbella turgidula Grunow, in Schmidt, A. Schmidt's Atlas Diatom.-Kunde, Pl. 9, Figs 23–26, 1875.

Lectotype. Plate 9, Fig. 23 in Schmidt (1875), designated by Tuji (2007).

Type locality. Puerto Rico and Kahyenmathay.

Epitype. An individual on slide 1504 in the Grunow Collection in the Naturhistorisches Museum Wien (W), designated by Tuji (2007, Fig. 15).

Epitype locality. Puerto Rico.

Valves lanceolate, dorsiventral, dorsal margins broadly convex and ventral margin convex; ends barely protracted, narrowly rounded, or subcapitate; length 36.5–45.0 μm, breadth 10.8–13.3 μm, L/B ratio 3.2–3.4; axial area linear, arched, central area indistinct to rounded; striae 9–12 in 10 μm, becoming 11–17 toward ends, 1–3 isolated pores; 21–24 punctae in 10 μm.

Cymbella tropica Krammer

Figs 64–70

Cymbella tropica Krammer, Diatoms Europe 3: 61, Figs 44:1–10, 49: 12, 13, 2002.

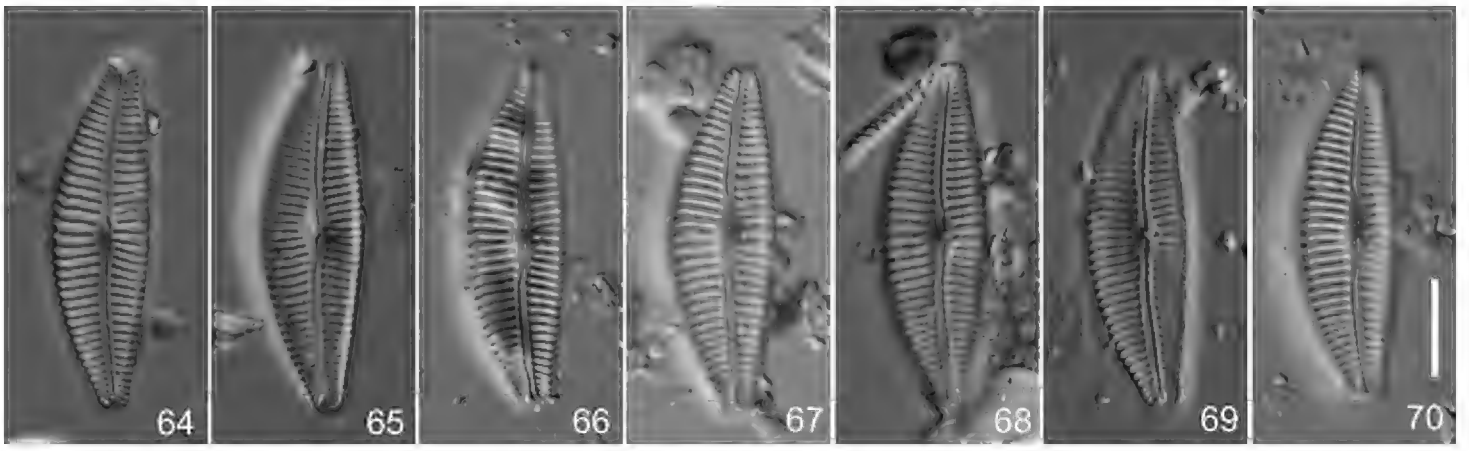
Holotype. Preparation 1015D IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany.

Lectotype (designated here). An individual on preparation 1015D IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany, represented by Fig. 64.

Type locality. Venezuela, Rio Manizanes, coll. Rumrich, 4.4.1990.

Valves lanceolate, dorsiventral, dorsal and ventral margins convex; ends barely protracted, rounded, or subcapitate, slightly deflected to ventral margin; length 34.5–42.7 μm, breadth 10.0–12.0 μm, L/B ratio 3.1–4.0; axial area linear, arched, central area indistinct to slightly rounded; striae 9–12 in 10 μm, becoming 11–12 toward ends, 1 isolated pore; 21–24 punctae in 10 μm.

Remarks. Krammer (2002) described *C. tropica* and recorded differences in the size, length/breadth ratio, and the presence of only one stigma as consistent diagnostic differences between this species and *C. turgidula*. Tuji (2007) recorded the occurrence of specimens of *C. turgidula* with 1–3 isolated pores, which was also observed by Krammer (2002) in his material. We observed a continuum between the metric characteristics of *C. tropica* and *C. turgidula*, even in those characters that were considered by Krammer (2002) as differentiating. However, the outline was more lanceolate, the ends more protracted and slightly deflected to the ventral side, and the degree of dorsiventrality was lower in *C. tropica* compared to *C. turgidula*.



Figures 64–70. *Cymbella tropica* Krammer from Venezuela in preparation 1015D IOK, holotype **64** Lectotype, designated here. Scale bar: 10 μm.

Cymbella subturgidula Krammer

Figs 71–97

Cymbella subturgidula Krammer, Diatoms Europe 3: 166, Figs. 44: 19–21, 2002.

Synonyms. = *Cymbella turgidula* var. *nipponica* Skvortzov, Philipp. J. Sci. 61: 283, Figs. 2:8, 4:4, 1936. (Figs 78–97)

– *Cymbella uenoi* Skvortsov in Skvortsov & Noda, Sci. Rep. Niigata Univ., ser. D (Biol.) 8: 19, Pl. 3, Fig. 3, 1971. (nom. inval.)

= *Cymbella rheophila* Ohtsuka in Ohtsuka & Tuji, Phycol. Res. 50: 245, Figs 7, 8, 2002.

= *Cymbella uenoi* Skvortsov *ex* Tuji, Diatom 23: 50, Figs 20–25, 2007.

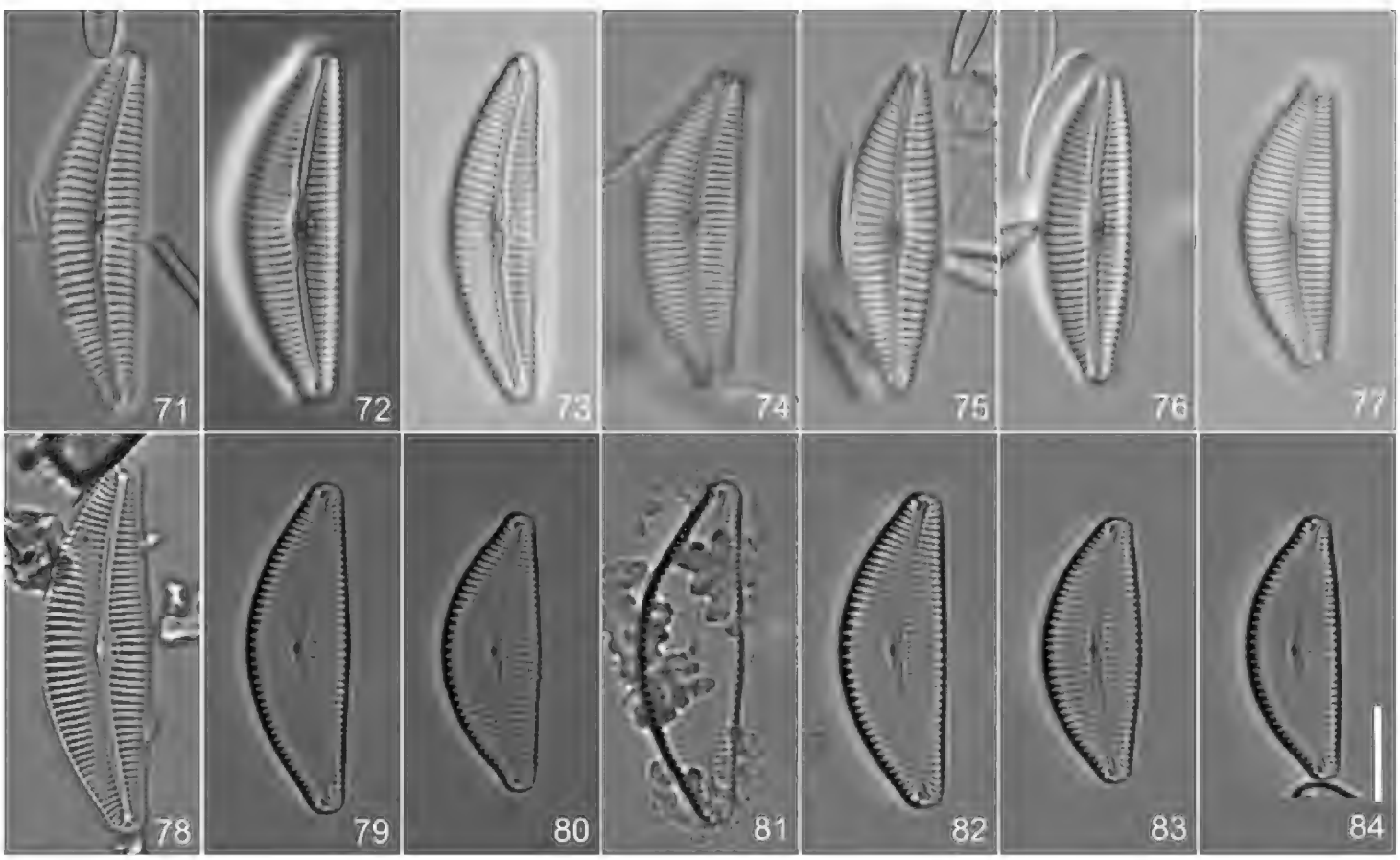
= *Cymbella uenoi* f. *nipponica* (Skvortsov) Tuji, Diatom 23: 54, Fig. 26, 2007.

Holotype. Preparation 1046E IOK [not "1046c IOK"], in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany.

Lectotype (designated here). An individual on preparation 1046e IOK, in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), Bremerhaven, Germany, represented by Fig. 73.

Type locality. Korea, Ulchin County, Kyungsang Pukdo, Kwangchun River.

Valvae slightly lanceolate to lanceolate, dorsiventral, dorsal margin broadly convex and ventral margin straight to convex; ends barely protracted, subrostrate to broadly subcapitate; length 26.3–41.0 μm, breadth 9.0–13.5 μm, L/B ratio 2.3–3.6; axial area linear to linear-lanceolate, arched, central area indistinct to slightly rounded; striae 9–13 in 10 μm, becoming 12–15 toward ends, 1–3 isolated pores; 21–26 punctae in 10 μm. In SEM, the striae showed lineolate punctae externally and internally, the striae are composed by an alveolus internally, surrounded by thick costae; the isolated pores are rounded externally; internally, the alveoli of the isolated pores are irregularly obovate and connected to intercostae, the margins with tooth-like structures (brocca); one apical pore field (APF) not divided by the external terminal fissure of the raphe can be observed on each pole of the valvae; the terminal nodule extends to the dorsal side,

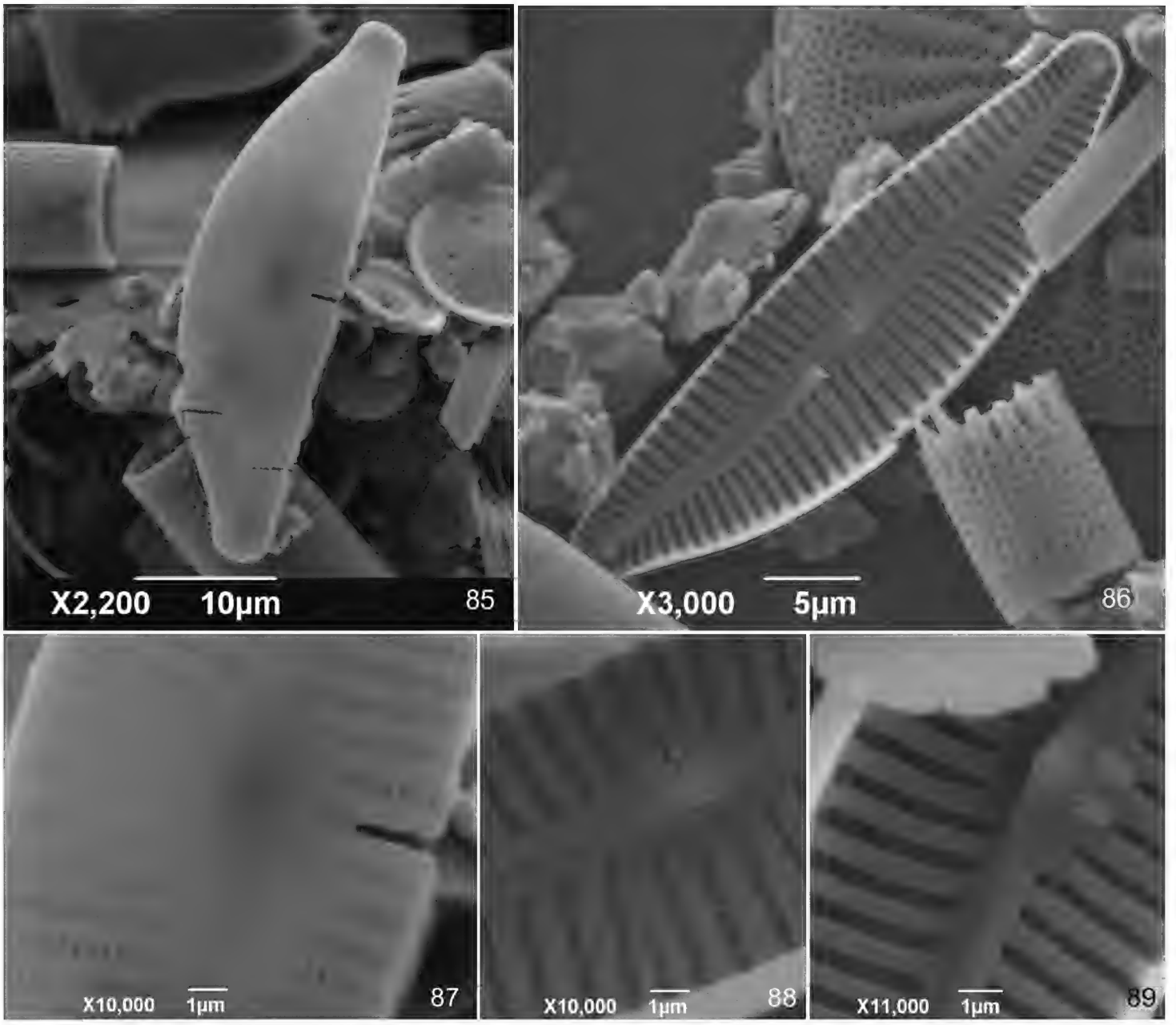


Figures 71–84. *Cymbella subturgidula* Krammer **71–77** Specimens from Korea in preparation 1046E IOK, holotype **73** Lectotype, designated here **78–80** Specimens from Japan in preparation R 214.928 **78**Isolectotype, designated here, of *C. turgidula* var. *nipponica* Skvortsov [≡ *C. rheophila* Ohtsuka], heterotypic synonym of *Cymbella subturgidula* **81** Specimen from Japan in preparation R 214.930 **82–84** Specimens from Japan in preparation R 214.932. Scale bar: 10 μm.

under the APF and has a short branch that penetrates the APF apically; the helictoglossae lie under the terminal nodule and are deflected to the dorsal side.

Remarks. Krammer (2002) described *C. subturgidula* based on preparation 1046c, which he designated as the holotype. This preparation was sought in the Alfred-Wegener-Institut für Polar- und Meeresforschung (BRM), where the entire Krammer Collection was transferred. However, preparation 1046c IOK is from Argentina in South America, and not from the holotype designated from Korea. In the protologue of *C. subturgidula*, Krammer (2002, p. 278 and 279) illustrated three specimens from preparation 1046E IOK, from Korea. Thus, the existence of slide 1046c IOK from Argentina, which is incongruent with the type locality, and the existence of slide 1046E which was used by Krammer to illustrate *C. subturgidula*, led us to consider the indication of preparation 1046c IOK as a typographical error.

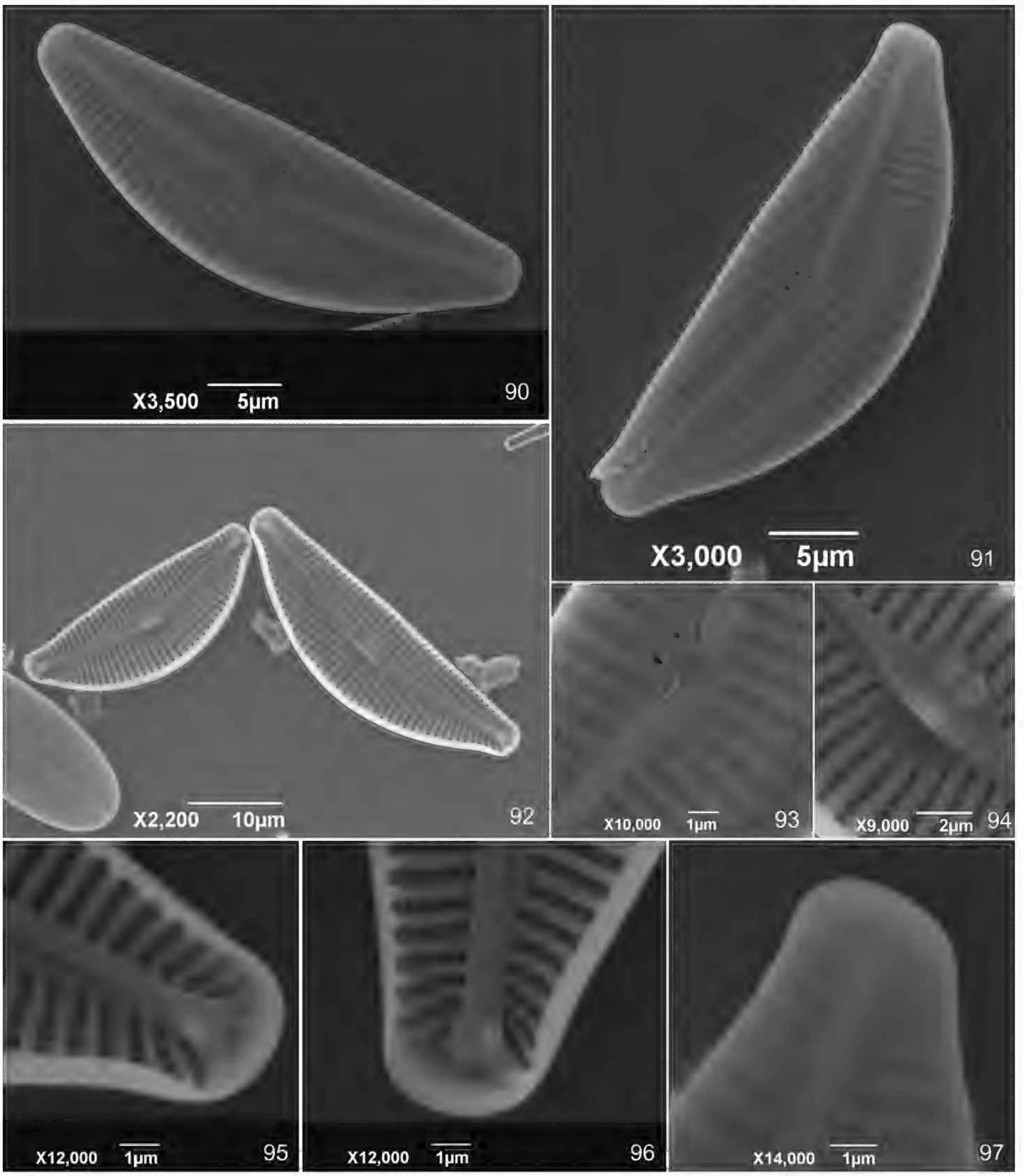
Cymbella subturgidula and *C. turgidula* are closely related species. However, *C. turgidula* is more lanceolate and has a higher degree of dorsiventrality than *C. subturgidula*. Moreover, *C. turgidula* is slightly broader than *C. subturgidula*, with a more prominent ventral side of the valve. The ends in the two species are different, being subrostrate-rounded in *C. turgidula* and slightly subrostrate-truncate in *C. subturgidula*. The central area is more distinct in *C. turgidula* than *C. subturgidula*. Although the number of punctae in 10 μm is the same in both species, the striae in *C. subturgidula* seem to be more coarsely punctuated than in *C. turgidula*.



Figures 85–89. *Cymbella subturgidula* Krammer from Japan, in the original material of *C. tumidula* var. *nipponica* Skvortsov [≡ *C. rheophila* Ohtsuka], in sample 0983 **85, 87** External valvar view **86, 88, 89** Internal valvar view.

Cymbella turgidula var. *nipponica* was described by Skvortzow (1936). He considered that this taxon differed from the nominate variety due to the elongated valve, slightly undulate ventral margin, and broad rostrate ends. Ohtsuka and Tuji (2002) proposed that maintaining this taxon as a variety of *C. turgidula* was not appropriate. They based their arguments on the co-occurrence of the nominate variety and the variety *nipponica*. Therefore, they proposed the name *C. rheophila* T.Ohtsuka for this taxon at the specific rank.

Skvortsov and Noda (1971) described *C. uenoi* Skvortsov, but did not indicate any type. According to Article 40.1 of the ICN, names of new genera or taxa of lower ranks published after 1958 are valid only when the type is indicated (McNeill et al. 2012), and therefore *C. uenoi* is invalid. Tuji (2007), however, indicated a type for *C. uenoi*, fulfilling the conditions required by the ICN. Therefore, the author of the name becomes *C. uenoi* Skvortsov *ex* Tuji. Tuji (2007) also transferred *C. turgidula* var. *nipponica* [≡ *C. rheophila*] to that species, resulting in the name *C. uenoi* f. *nipponica*, considering erroneously that the name *C. uenoi* had priority under the name *C. rheophila*.



Figures 90–97. *Cymbella subturgidula* Krammer from Japan in sample 1093 **90, 91, 93, 97** External valvar view **92, 94, 95, 96** Internal valvar view.

Tuji (2007, p. 54) suggested the conspecificity of *C. subturgidula* and *C. uenoi*. The observations of the type material of *C. uenoi* provided by Tuji (2007) and *C. uenoi* f. *nipponica* [≡ *C. rheophila*], compared with the type material of *C. subturgidula* (i.e. morphometric characteristics), led us to agree with Tuji (2007). Since *C. uenoi* was validated only in 2007, the valid names of this species are either *C. rheophila* or *C. subturgidula*, both published in 2002, and not *C. uenoi* as stated by Tuji (2007). *C. subturgidula* was described in The Diatoms of Europe, volume 3, published by Kram-

mer on 28 January 2002 (Koeltz Scientific Books, pers. comm.), while *C. rheophila* was not published before 29 July 2002, the date of acceptance of the paper. Therefore, the epithet *subturgidula* has priority over the epithet *rheophila.*

The re-analysis of the type material of *C. subturgidula* allows us to broaden the metric data of this species compared to its original description. We observed a wider range of values for length (30.3–37.4 vs. 36–37 μm), breadth (9.0–12.8 vs. 10.0–11.0 μm), striae (9–11 vs. 10–11 in 10 μm) and punctae (21–24 vs. 24 in 10 μm) compared to Krammer's (2002) description. Metric characteristics of the original material of *C. turgidula* var. *nipponica* [≡ *C. reophila*; ≡*C.uenoi* f. *nipponica*, isolectotype designated here (Fig. 78)] and *C. uenoi* (Table 1) also agree with the characteristics of the type population of *C. subturgidula* (Skvortzov and Noda 1971, Ohtsuka and Tuji 2002, Tuji 2007).

Krammer (2002) described two isolated pores in *C. subturgidula*. Similarly, Ohtsuka and Tuji (2002) and Tuji (2007) observed two isolated pores in the material of *C. turgidula* var. *nipponica* [≡ *C. rheophila*; ≡ *C. uenoi* f. *nipponica*] and *C. uenoi*. However, in the original sample of the lectotype of *C. turgidula* var. *nipponica* we found some specimens with only one isolated pore (Fig. 88), and in recent material collected in Lake Biwa we found up to three isolated pores (Fig. 90).

Discussion

Few studies have discussed the criteria to delimit infraspecific ranks in diatoms. Cox (1997) reviewed this issue and found contradictions among the criteria adopted by different researchers over time. In order to resolve this question, she proposed a pragmatic solution to delimit infraspecific taxonomic ranks. According to Cox (1997), the term variety should be used "for populations (within the same species) which are ecologically and morphologically distinct, in which there is no evidence of morphological intergrading under intermediate conditions".

However, taxonomic analyses of some species complexes in diatoms using molecular data have demonstrated that Cox's (1997) suggestions about the delimitation of varieties do not apply to every situation. *Sellaphora pupula* (Kütz.) Mereschk. sensu lato and *Nitzschia palea* (Kütz.) W.Sm. sensu lato, for example, have been shown to be an assemblage of pseudocryptic species that correspond, in the majority of instances, to populations with intergrading morphological characteristics (Mann et al. 2008; Trobajo et al. 2009). We have found a similar situation in populations that were clearly identified as *Cymbella tumida* (Bréb.) Van Heurck (unpublished data).

The criteria adopted by Krammer (2002, 2003) to delimit several specific and infraspecific taxa of *Cymbella* take into account variations between the type and similar individuals from other populations. It is important to note that, for Krammer (2002, 2003), types can be individuals marked or represented in some illustration, as well as a group of individuals mounted in a preparation, as defined in the ICN (McNeill et al. 2012).

Because of the lack of taxonomic studies on the *Cymbella affinis*/*tumidula*/*turgidula* species complex using molecular data, we opted to use Krammer's concept in order to attempt to organize this confusing group. This criterion is usual as a reference to circumscribe groups with similar morphologies, even if individuals with these morphologies sometimes overlap. Some authors prefer to define similar groups as "morphodemes", which have no nomenclatural status. However, in some cases, this definition sounds more similar to the old taxonomy when any taxonomic unit could be denominated by different names, but scientifically by a long sentence that is more similar to the current "diagnosis". Although the concept of varieties used here implies the publication of nomenclatural novelties, none of them are new proposals, but rather are simply adjustments of already existing names that are in confusing combinations, because of a misinterpretation by Krammer (2002) as well as the history of the taxa and the evolution of nomenclatural rules.

The criteria of delimitation of taxa and the weight of characters in diatoms are variable from group to group (Mann 1999). While in some groups the density of striae is a good morphological indicator of different species, in other groups this can be irrelevant (Abarca et al. 2014). Different sizes and valve outlines are not always good characters to delimitate taxa such as in the complex *Encyonema silesiacum*/*minutum*/*ventricosum* (unpublished data). Thus, it is common to find taxa with polythetic definitions, that is, in which a set of characteristics, sometimes interweaving with other taxa, are taken into account (Needham 1975). The delimitation of *Cymbella* taxa is a clear example of polythethism (Krammer 2002).

In this context, several characters must be considered in their characterization and identification of taxa. The degree of dorsiventrality of the valve, for example, is slightly higher in *C. affinis* than in *C. tumidula*, and even higher in *C. schilleri* Krammer (2002, Fig. 26: 7, 8) than in *C. orientalis* Lee (Krammer 2002, Fig. 26: 1–6). Another example is the thickness of the striae. The striae of *C. tumidula* are narrower than in individuals of *C. affinis* in LM, even in specimens of the same size. This is often the result of thick intercostae and the size of areolae, which can only be observed in SEM (Krammer 2002, Figs 5: 1, 23: 18). Thus, these characters, alone, seem to be unimportant but in combination with further features can provide a better concept of the taxa.

Ultrastrutural characterizations by SEM are also important in diatoms, but not always possible using the original material. Records from the literature have demonstrated that representatives of the *C. affinis*/*tumidula*/*turgidula* species complex present similar internal ultrastructure of the isolated pores (i.e., aperture covered by small teeth) and the intercostae. This is the case of *C. affinis* (=*C. excisa* sensu Krammer 2002, Figs 5: 1), *C. affinis* var. *subcapitata* (Krammer 2002, Figs. 10: 16–18) *C. tumidula* var. *tumidula* (=*C. affinis* sensu Krammer 2002, Fig. 23: 18), and *C. subturgidula* (Figs 89, 94), which are similar to *C. cymbiformis* C.Agardh, type of the genus (Krammer 2002, Figs 5: 2, 3). However, the internal structure of isolated pores and the intercostae of these taxa are different to other species such as *C. aspera* (Ehrenb.) Perag. (Krammer 2002, Fig. 5: 4), *C. neolanceolata* W.Silva (=*C. lanceolata* sensu Krammer 2002, Fig. 5:5), *C. neocistula* Krammer (2002, Figs 5: 6, 7). This demonstrates that the complex

C. affinis/*tumidula*/*turgidula* is morphologically closer related to the type of the genus, than to the other species of the same genus.

The economic and ecological uses of diatoms require a refined taxonomy, which is more detailed than simply species complexes. This is especially true for bioassessments using diatoms (European Committee for Standardization 2003). *Gomphonema lagenula* Kütz. has for a long time been treated as synonym of *G. parvulum* Kütz., since the variability in the shape of the ends were considered insufficient to distinguish the taxa (VanLandingham 1971). However, differences in the ecological preferences of these two taxa were recorded and the independence of the two species has been confirmed by molecular data (Kermarrec et al. 2013, Abarca et al. 2014). Differences between the ecological preferences of *Nitzschia palea* (Kütz.) W.Sm. var. *palea* and *N. palea* var. *debilis* (Kütz.) Grunow have also been recorded, and simply morphological characters are insufficient to separate the series of phenotypic expressions subscribed to *N. palea* (Trobajo et al. 2009). Thus, the efficiency in the use of diatoms in activities such as bioassessments needs accuracy and taxonomic harmonization (Manoylov 2014). But this will only be possible if important characteristics such as ecological preferences can be permanently attached to a taxon or an accessible designation, which is facilitated by the establishment of correct types and by the knowledge of these types.

Naturally, beside the knowledge of types, supplementary studies are necessary to record phenotypic plasticity resulting from different ecological conditions or by life cycles. Such studies should be carried out in natural or cultivated samples in order to observe a more realistic concept of the species (Mann 1999). These studies will allow us to observe slight changes in the morphology of the valves (i.e., outline, measures, etc.) such as some of those observed in the type population of the taxa discussed here. Moreover, these studies can possibly verify the relationship among morphological characters, which are associated in a polythetic way during the establishment of specific concepts and in their use in the identification of taxa of similar groups.

Conclusion

The process of lectotypification can markedly influence the identity of some taxa and can sometimes substantially change the relation to other taxa. The designation of a type for *C. affinis* resulted in a profound restructuring of *C. affinis*, *C. excisa* and *C. tumidula*. *C. excisa* has been shown to be the same taxon at the species level as *C. affinis*, but because of its specific morphology is treated herein at a different rank. The epithet *affinis* has priority over the epithet *excisa*, as defined by the criterion of the first effective publication. Thus, four infraspecific taxa of *C. excisa* were transferred to *C. affinis*.

The lectotypification of *C. tumidula* Grunow and comparisons with the lectotype of *C. affinis* allowed us to conclude that the two species are independent. *C. affinis* var. *procera* was treated as a new species, which is closer to *C. tumidula* than *C. affinis* because of morphological similarities. Infraspecific taxa described by Krammer (2002) within *C. affinis* had small differences in relation to the type of *C. tumidula*, and they are recombined herein.

The analysis of the type and the history of taxa such as *C. subturgidula* Krammer, *C. rheophila* Ohtsuka, and *C. uenoi* Skvortsov *ex* Tuji allowed us to conclude that these taxa are conspecific, and to determine that the epithet *subturgidula* has priority.

Acknowledgments

The authors thank Jovita Yesilyurt, from the Natural History Museum, London, and Taisuke Ohtsuka, from the Lake Biwa Museum, Kusatsu, Shiga Prefecture, who provided the material for analysis; and the Laboratório de Microscopia Eletrônica do Museu Nacional for the SEM analyses. The first author is grateful to CAPES and DAAD/CAPES for the award of a doctoral scholarship and a "sandwich" scholarship for study at the BGBM, Berlin, Germany.

References

Abarca N, Jahn R, Zimmermann J, Enke N (2014) Does the cosmopolitan diatom *Gomphonema parvulum* (Kützing) Kützing have a biogeography? PLoS ONE 9: e86885. doi: 10.1371/journal.pone.0086885

Cleve PT (1894) Synopsis of the Naviculoid Diatoms, Part I. Konglische Svenska Vetenskaps-Akademiens Handlingar, ser. 4 26(2): 1–216.

Cox EJ (1997) Assessing and designating diatom taxa at or below the species level - a consideration of the current status and some suggested guidelines for the future. Nova Hedwigia 65(1-4): 13–26.

European Committee for Standardization (2003) Water quality - Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers. European Committee for Standardization, Brussel, 14.

Grunow A (1882) Beiträge zur Kenntniss de fossilen Diatomeen Österreich-Ugarns. Beiträge zur Paläontologie Österreich-Ugarns und des Orients 2: 136–159.

Kermarrec L, Bouchez A, Rimet F, Humbert J-F (2013) First evidence of the existence of semi-cryptic species and of a phylogeographic structure in the *Gomphonema parvulum* (Kützing) Kützing complex (Bacillariophyta). Protist 164: 686–705. doi: 10.1016/j.protis.2013.07.005

Krammer K (2002) Diatoms of the European inland waters and comparable habitats. *Cymbella*. In: Lange-Bertalot H (Ed) Diatoms of Europe 3. A.R.G. Gantner Verlag KG, Ruggel, 1–584.

Krammer K (2003) Diatoms of the European inland waters and comparable habitats. *Cymbopleura*, *Delicata*, *Navicymbula*, *Gomphocymbellopsis*, *Afrocymbella*. In: Lange-Bertalot H (Ed.) Diatoms of Europe 4. A.R.G. Gantner Verlag KG, Ruggel, 1–529.

Krammer K, Lange-Bertalot H (1986) Bacillariophyceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer H (Eds) Süsswasserflora von Mitteleuropa 2/1. Gustav Fischer Verlag, Stuttgart, Jena, 1–876.

Kützing FT (1844) Die kieselschaligen Bacillarien oder Diatomeen. W. Köhne, Nordhausen, 1–152. doi: 10.5962/bhl.title.64360

Mann DG (1999) The species concept in diatoms. Phycological Research 38: 437–495. doi: 10.2216/i0031-8884-38-6-437.1

Mann DG, Thomas SJ, Evans KM (2008) Revision of the diatom genus *Sellaphora*: a first account of the larger species in the British Isles. Fottea 8(1): 15–78. doi: 10.5507/fot.2008.002

Manoylov KM (2014) Taxonomic identification of algae (morphological and molecular): species concepts, methodologies, and their implications for ecological bioassessment. Journal of Phycology 50: 409–424. doi: 10.1111/jpy.12183

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'Homme Van Reine WF, Smith GF, Wiersema JH, Turland NJ (2012) International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Koeltz Scientific Books, Königstein, 1–240. [Regnum Veg. 154].

Needham R (1975) Polythetic classification: convergence and consequences. Man, New Series 10: 349–369. doi: 10.2307/2799807

Ohtsuka T, Tuji A (2002) Lectotypification of some pennate diatoms described by Skvortzow in 1936 from Lake Biwa. Phycological Research 50: 243–249. doi: 10.1046/j.1440-1835.2002.00281.x

Patrick R, Reimer CW (1975) The diatoms of the United States. Volume 2, Part 1. Monographs of the Academy of Natural Sciences of Philadelphia 13: 1–213.

Schmidt A (1875) Atlas der Diatomaceenkunde, Series I 3. O.R. Reisland, Leipzig, 9–12.

Skvortzov BV, Noda M (1971) On recent and fossil freshwater diatoms from Japan II. Science Reports of the Niigata University, Biology Series D 8: 13–27.

Skvortzow BW (1936) Diatoms from Biwa Lake, Honshu Island, Nippon. Philippine Journal of Science 61: 253–296.

Trobajo R, Clavero E, Chepurnov VA, Sabbe K, Mann DG, Ishihara S, Cox EJ (2009) Morphological, genetic and mating diversity within the widespread bioindicator *Nitzschia palea* (Bacillariophyceae). Phycologia 48: 443–459. doi: 10.2216/08-69.1

Tuji A (2007) Type examination and typification of *Cymbella affinis* Kütz., C. *turgidula* Grunow and *C. uenoi* Skvortsov. Diatom 23: 49–54. https://www.jstage.jst.go.jp/article/diatom1985/23/0/23_49/_pdf

VanLandingham SL (1971) Catalogue of the fossil and recent genera and species of diatoms and their synonyms. Part 4. *Fragilaria* through *Naunema*. J. Cramer, Lehre.